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Structure activity relationships of furanones, dihydropyrrolones and thiophenones as potential quorum sensing inhibitors

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Abstract

Since their initial isolation from the marine alga *Delisea pulchra*, bromofuranones have been investigated as potential inhibitors of quorum sensing in various bacterial strains. Quorum sensing is an important mechanism by which bacteria co-ordinate their molecular response to the environment. Importantly, quorum sensing is intrinsically linked to bacterial antibiotic resistance. Inspired by nature, chemists have developed a wide variety of synthetic analogues in an effort to elucidate the structure activity relationships of these compounds, and to ultimately develop novel antimicrobial agents. In this work, we describe advances in this field while paying particular attention to apparent structure activity relationships. This review is organised according to the main ring systems under investigation, namely furanones, dihydropyrrolones and thiophenones.

Keywords

Quorum sensing, resistance, furanones, dihydropyrrolones, thiophenones

1. Introduction

The discovery of penicillin by Alexander Fleming in 1928 sparked the beginning of an antibiotic revolution that transformed modern medicine [1]. Arguably the most significant development in the history of medicine, antibiotics revolutionised the treatment of infectious diseases. Antibiotics allowed life-threatening infections such as pneumonia, scarlet fever, cholera and tuberculosis to be treated, saving countless lives. Unfortunately, the phenomenon of antibiotic resistance now

threatens the efficacy of antibiotics. Although antibiotic resistance occurs naturally as bacteria evolve, the process has been greatly accelerated by the extensive overuse and misuse of antibiotics, the discovery of which cannot compete with the pace of bacterial resistance. This phenomenon has paved the way for the development of novel, non-antibiotic treatments for bacterial infections.

Over the past two decades compounds that interfere with microbial quorum sensing (QS) and biofilm formation have been extensively investigated as potential routes to the next generation of antimicrobials [2-5]. QS is a bacterial cell-cell communication mechanism that allows bacteria to regulate gene expression in response to changes in population density [6]. Bacteria that engage in QS produce diffusible signalling molecules called autoinducers (AIs) which act as chemical messengers between bacteria of the same or different species [6, 7]. At high bacterial population densities, critical concentrations of signalling molecules are produced which bind to their cognate receptors, allowing bacteria to adapt rapidly to their environment *via* the up-regulation of virulence genes and other loci [8]. This results in the production of virulence factors, toxins and antibiotic tolerant biofilms in response to a flux in population density.

QS, previously known as “autoinduction”, was first observed in *Vibrio fischeri*, a luminescent marine bacterium in the late 1960s [9, 10]. Hastings and Nealson discovered that these bacteria only bioluminesce when they reach high population densities, and hypothesised that the process is likely regulated by the secretion and movement of molecules between bacterial cells [11]. Further investigation confirmed this hypothesis and the peptide involved in *Vibrio fischeri* quorum sensing was identified as *N*-acyl homoserine lactone (AHL) [12]. QS is used by both Gram-positive and Gram-negative bacteria. Different bacterial species regulate quorum sensing using different classes of autoinducers [8, 13]. The signalling molecules produced most commonly in Gram-positive and Gram-negative bacteria are oligopeptides and AHLs respectively, while a universal signalling molecule, autoinducer-2 (AI-2), may be present in both bacterial classes (Figure 1) [3].

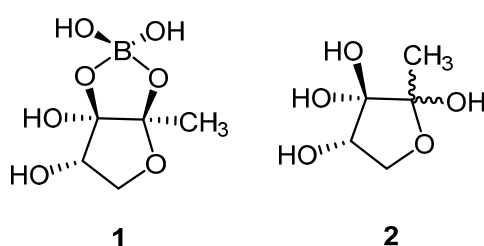


Figure 1. Structure of boron-containing AI-2 (**1**), produced by *Vibrios* and boron-free AI-2 (**2**), produced by *Salmonella* [14].

AI-2 is produced by both Gram-positive and Gram-negative bacteria and is a universal signalling molecule for interspecies communication [14, 15]. Methyltransferases, 5'-methylthioadenosine (MTAN) and LuxS enzymes catalyse the conversion of S-adenosylmethionine (SAM) to 4,5-dihydroxy-2,3-pentanedione (DPD), an unstable compound which can spontaneously cyclise to produce multiple DPD derivatives, known as AI-2 molecules. Crystal structures of AI-2 from the marine bacterium *Vibrio harveyi*, identify the autoinducer as a bicyclic furanosyl borate ester (**1**) bound to its sensor protein LuxP [11]. The structure consists of two five-membered rings, fused together, with one of the rings containing a boron atom which bridges the diester. AI-2 may also adopt a monocyclic structure (**2**) which lacks the boron atom in bacteria such as *Salmonella typhimurium*. Bacteria can detect endogenous AI-2 as well as AI-2 produced by other bacterial species due to the interconversion of AI-2 molecules [15]. Several AI-2 receptors have been identified to date and vary depending on the bacteria under investigation [16].

Although QS is a process that is widely utilised by many bacterial species, the survival of quorum sensing mutants has shown that it is not an essential process [17]. Thus, quorum sensing inhibitors (QSI) have the potential to act as novel antimicrobial agents in the treatment and management of microbial infections. The ability of these compounds to inhibit QS without suppressing bacterial growth reduces the likelihood of the bacteria developing resistance. The reduction of bacterial QS has been shown to significantly lower virulence factor production and opens up the possibility that QSIs may constitute a novel class of non-bactericidal and non-bacteriostatic antimicrobial agents [18].

Halogenated furanones, originally isolated from a marine alga, have proven to be highly effective inhibitors of AI-2 [19]. It is believed that they act as potent Michael acceptors and bind with the DPD synthase, LuxS, blocking the production of AI-2 [20]. Since that initial discovery, many groups have investigated a variety of halogenated furanones as well as related dihydropyrrolones and thiophenones as potential QSIs. In this review, we summarise progress to date and discuss the structure activity relationships apparent from this work.

2. Furanones

The marine alga *Delisea pulchra*, native to the south eastern coast of Australia, produces approximately thirty different halogenated furanone compounds [19]. In 1993 de Nys and co-workers identified seven new furanones (Table 1) along with seventeen previously isolated furanones from this algae (Table 2). These compounds, which vary in side chain structure,

substitution at the 4-position and in the number and nature of the halogen substituents, inhibit colonisation of the algae surface [19, 21]. Importantly, the formation of bacterial biofilms is also inhibited by these secondary metabolites [22].

Manefield *et al.* investigated the five most abundant *D. pulchra* furanones (Table 2, entries 1-3, 5, 8) as AHL QS antagonists in the bioluminescent monitor strain *E-coli* MT102(pSB403) and compared them to synthetic furanone **3** (Figure 2) [23]. The presence of a hydroxyl group on the C3-alkyl chain in **13** (entry 3) confers greater inhibitory activity than the acetoxy group in **15** or **19** (entries 5 and 9), which in turn is more active than a hydrogen atom. The presence of at least one halogen, typically a bromine atom on the exocyclic vinyl group, is necessary for efficacy. Furanone **19**, which contains an iodine atom, is marginally more active than bromine-substituted **15**. In the presence of furanones **13**, **15** and **19**, *E-coli* bioluminescence was reduced from >100,000 relative light units (RLUs) to less than 1,000 RLUs. The reduction in RLUs highlights the potential of furanones to act as antagonists of the AHL QS system. These naturally occurring compounds were more active than furanone **3**, which saw a luminescence output of >10,000 RLUs. None of the compounds affected the bacterial planktonic growth at the concentrations tested. Given the significant differences in activity observed when even small changes are made to the structures of the naturally occurring furanones, a number of research groups have subsequently sought to develop analogues which are more potent and less toxic.

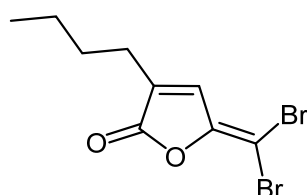
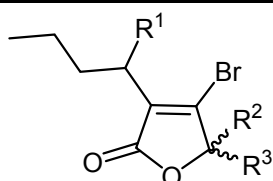


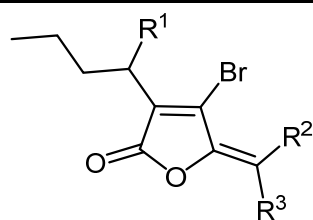
Figure 2. 3-Butyl-5-(dibromomethylene)furan-2(5H)-one, **3**

Table 1. Novel furanones isolated from *Delisea pulchra*.



Entry	Compound	R ¹	R ²	R ³
1	4	OAc	OCH ₃	CH ₂ I
2	5	OH	CH ₂ I	OCH ₃
3	6	OH	OCH ₃	CH ₂ I
4	7	OAc	CH ₃	OCH ₃
5	8	OAc	OCH ₃	CH ₃
6	9	OAc	CHBr ₂	OCH ₃
7	10	OAc	OCH ₃	CH ₂ I

Table 2. Previously reported furanones isolated from *Delisea pulchra*.

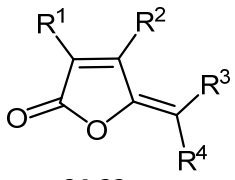
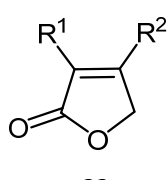


Entry	Compound	R ¹	R ²	R ³
1	11	H	Br	Br
2	12	H	H	Br
3	13	OH	H	Br
4	14	OH	Br	H
5	15	OAc	H	Br
6	16	OAc	Br	H
7	17	OH	H	I
8	18	OH	I	H
9	19	OAc	H	I
10	20	OH	Br	Br
11	21	OAc	Br	Br

12	22	OH	H	Cl
13	23	OH	Cl	H

A library of brominated furanones was synthesised by Janssens *et al.* and their QS inhibitory activity against *Salmonella enterica* serovar *typhimurium* was determined (Table 3) [24]. Compounds which lacked an alkyl chain at the C3 position were the strongest biofilm inhibitors with furanones **24**, **25** and **26** having IC₅₀ values of 15 ± 5 µM (entry 1), 15 ± 4 µM (entry 2) and 10 ± 3 µM (entry 3) respectively. These molecules were also found to be more toxic to *Salmonella* than the alkylated derivatives, decreasing bacterial growth at low concentrations (30 – 40 µM) and inhibiting growth completely at 500 µM concentrations. Janssens attributed this finding to the higher aqueous solubility of non-alkylated **24-26**. Only three of the alkylated furanones exhibited QS inhibitory activity, namely **28**, **12** and **29**, recording IC₅₀ values of 50 ± 5 µM (entry 5), 100 ± 10 µM (entry 7) and 60 ± 15 µM (entry 8). **28** and **29** influenced bacterial growth at concentrations greater than 150 µM. By contrast, monobrominated furanone **12** did not impact planktonic growth even at higher concentrations. Interestingly, **28**, **12** and **29** show greater potential as QSIs than the more active candidates **24-26**, due to their better toxicity profiles. **22** (entry 4) and **11** (entry 6), which feature a dibromomethylene group, were devoid of activity. Similarly, furanones **30-32** with alkyl chains of greater than six carbons displayed no inhibition (entries 9-11), most likely due to the insufficient aqueous solubility. The absence of any QS effect for non-halogenated furanone **33** (entry 12) highlights the significance of the bromine atom. Overall, molecules with ethyl, butyl and hexyl side chains inhibited biofilm formation without impacting bacterial growth, while longer chains at the C3 position led to no reduction in biofilm formation being observed [24, 25]. Candidates which lacked a 3-alkyl chain were generally identified as being toxic to planktonic cells.

Table 3. Effect of halogenated furanones on *Salmonella enterica* serovar *typhimurium*.

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>24-32</p> </div> <div style="text-align: center;">  <p>33</p> </div> </div>						
Entry	Compound	R ¹	R ²	R ³	R ⁴	IC ₅₀ (µM)

1	24	H	H	H	Br	15 ± 5
2	25	H	H	Br	Br	15 ± 4
3	26	H	Br	H	Br	10 ± 3
4	27	CH ₂ CH ₃	H	Br	Br	NA
5	28	CH ₂ CH ₃	Br	H	Br	50 ± 5
6	11	(CH ₂) ₃ CH ₃	Br	Br	Br	NA
7	12	(CH ₂) ₃ CH ₃	Br	H	Br	100 ± 1
8	29	(CH ₂) ₅ CH ₃	Br	H	Br	60 ± 15
9	30	(CH ₂) ₇ CH ₃	Br	H	Br	NA
10	31	(CH ₂) ₉ CH ₃	Br	H	Br	NA
11	32	(CH ₂) ₁₁ CH ₃	Br	H	Br	NA
12	33	(CH ₂) ₃ CH ₃	H	-	-	NA

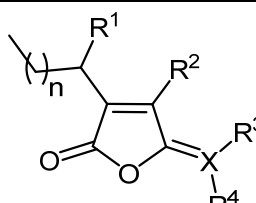
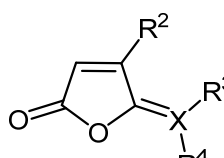
NA = No activity at 1 mM concentration (highest concentration tested)

On foot of Janssens' work, Steenackers and co-workers investigated the influence of the bromination pattern of the furanone ring structure on activity against *S. typhimurium* ATCC14028 (Table 4) [24, 25]. Homologues **34-36**, which differ only in the length of the 3-alkyl chain, exhibit similar IC₅₀ values of 10.74 – 23.12 µM in *S. typhimurium* (entries 1-3). All of these examples contain a monobrominated methylene group and an additional bromine on the 4-position of the ring. Furanone **36**, which bears a hexyl chain (entry 3), possesses slightly better activity than analogues bearing shorter propyl (entry 1) or butyl (entry 2) substituents at the C-3 position. **34-36** are significantly more active than **40-42** which lack the C4 bromine and instead feature a dibrominated methylene group with IC₅₀ values in the range of 148 µM – 199.9 µM (entries 7-9). A bromine atom at the 4-position was deemed critical for biofilm inhibition, with **47** (entry 14), found to be a potent biofilm inhibitor (IC₅₀ of 19.42 µM) of *S. typhimurium*. Analogue **46**, which contains a single bromine atom on the exocyclic vinyl and no bromine at the 4-position, was inactive against *S. typhimurium* at the highest tested concentration (entry 13). The concentrations required to inhibit biofilm formation by 50% had no effect on the planktonic bacterial growth of *S. typhimurium*.

In a related study, the same group also examined the effect of furanones on *V. harveyi* bioluminescence (Table 4). The non-alkylated furanones **24-26** (entries 25-27), in addition to 3-ethyl substituted **34** (entry 1) and **43** (entry 10), reduced *V. harveyi* bioluminescence by 50% at low concentrations (IC_{50} of 1.152 μ M – 9.268 μ M), while also inhibiting growth at comparable concentrations. Similar to previous observations with *S. typhimurium*, monobromomethylene-substituted **34-36** (entries 1-3) were more potent inhibitors of *V. harveyi* bioluminescence than dibromomethylene derivatives **40-42** (entries 7-9). Analogue **39**, which contains a dodecyl side chain at the 3-position, did not influence either *V. harveyi* bioluminescence or planktonic growth even at higher concentrations (entry 6). Surprisingly, the C4-brominated maleic anhydride derivative **48** (entry 15), which lacks an exocyclic methylene, did inhibit bioluminescence (IC_{50} = 10.56 μ M).

Janssens also concluded that bromination of the alkyl chain led to a significant increase in activity against both bacterial strains. A comparison of ethyl-substituted **43** with bromoethyl-substituted **54** reveals a reduction in IC_{50} from 57.56 μ M (entry 10) to 4.09 μ M (entry 21) in *S. typhimurium*. By contrast, the introduction of an acetoxymethyl group at the same position proved detrimental (entry 24).

Table 4. Effect of halogenated furanones on *Salmonella typhimurium* and *Vibrio harveyi* bioluminescence.

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>34-57</p> </div> <div style="text-align: center;">  <p>24-26</p> </div> </div>									
Entry	Compound	R ¹	R ²	R ³	R ⁴	X	n	<i>Salmonella typhimurium</i> IC_{50} (μ M)	<i>Vibrio harveyi</i> IC_{50} (μ M)
1	34	H	Br	H	Br	C	0	17.91	9.268
2	35	H	Br	H	Br	C	2	23.12	1.362
3	36	H	Br	H	Br	C	4	10.74	11.910
4	37	H	Br	H	Br	C	6	NA	3.414

5	38	H	Br	H	Br	C	8	NA	26.62
6	39	H	Br	H	Br	C	10	NA	NA
7	40	H	H	Br	Br	C	0	199.90	104.600
8	41	H	H	Br	Br	C	2	148.00	18.900
9	42	H	H	Br	Br	C	4	160.10	84.050
10	43	H	Br	Br	Br	C	0	57.46	NA
11	44	H	Br	Br	Br	C	2	NA	14.36
12	45	H	H	-	-	O	4	>1000	97.99
13	46	H	H	H	Br	C	4	>1000	NA
14	47	H	Br	H	H	C	4	19.42	1.357
15	48	H	Br	-	-	O	4	65.89	10.560
16	49	Br	Br	H	Br	C	0	4.50	6.339
17	50	Br	Br	H	Br	C	2	5.71	1.180
18	51	Br	Br	H	Br	C	4	3.23	5.207
19	52	Br	H	Br	Br	C	0	1.16	0.7742
20	53	Br	H	Br	Br	C	2	1.31	0.1999
21	54	Br	Br	Br	Br	C	0	4.09	2.761
22	55	OAc	Br	H	Br	C	2	131.30	50.05
23	56	OAc	H	Br	Br	C	0	161.60	NA
24	57	OAc	Br	Br	Br	C	0	7.29	6.311
25	24	-	H	H	Br	C	-	NA	4.071
26	25	-	H	Br	Br	C	-	NA	2.878
27	26	-	Br	H	Br	C	-	NA	2.708

NA = No reported activity

In their search for QSIs of *Pseudomonas aeruginosa*, Hentzer and co-workers identified **24** as a promising candidate (Figure 3) [26]. At a 28.5 μ M concentration, **24** reduced the expression of reporter strain lasB-gfp(ASV) by 40%, underlining the QS ability of **24** to inhibit the *las* QS system in

P. aeruginosa. This system is responsible for regulating gene expression in the bacterium [27]. The same group later reported that dibrominated derivative **25** also inhibited QS activity in *P. aeruginosa*, reducing both the production of extracellular virulence factors, as well as biofilm formation [21].

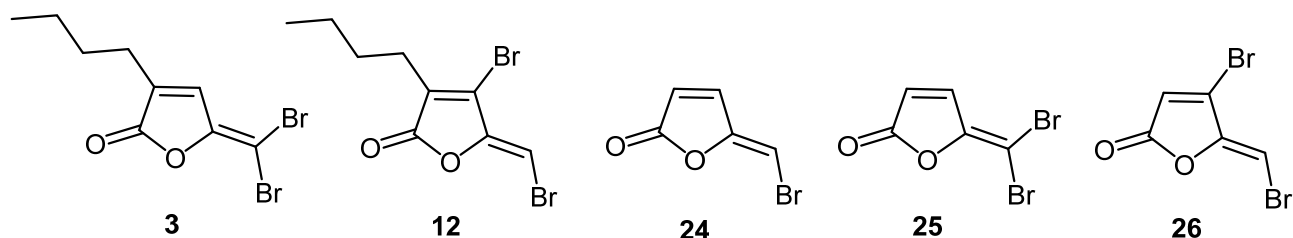


Figure 3. Promising QS inhibitors of *P. aeruginosa*

Han and colleagues screened a series of brominated furanones for potential inhibitors of *E. coli* (Table 5) [28]. Initial toxicity assays identified **61** (entry 4) and **62** (entry 5), which bear an exocyclic bromomethylene at C3, as being toxic at 5 µg/mL – 10 µg/mL concentrations and are, therefore, likely bactericidal to *E. coli*. Analogues **58-60** (entries 1-3), **63** (entry 6) and **64** (entry 7) had no impact on bacterial growth at concentrations up to 60 µg/mL. Of these, **58-60** were the most promising candidates and inhibited biofilm formation by 75% (entry 1), 63% (entry 2) and 80% (entry 3) respectively. These compounds all contain an exocyclic vinyl bromide confirming the biological significance of this motif. By contrast, furanone **63**, which does not feature a vinyl bromide group, was inactive (entry 6). Surprisingly, structurally similar furan **64** did display an inhibitory effect on biofilm formation, despite the absence of the exocyclic vinyl group (entry 7).

Table 5. Biofilm inhibitory activity against *Escherichia coli* at 60 µg/mL concentration.

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> 58-62 </div> <div style="text-align: center;"> 63 </div> <div style="text-align: center;"> 64 </div> </div>						
Entry	Compound	R ¹	R ²	R ³	R ⁴	Biofilm Inhibition
1	58	CH ₃	Br	H	Br	75%
2	59	CH ₃	H	Br	Br	63%

3	60	CH(Br) ₂	H	Br	Br	80%
4	61	CH ₂ Br	H	Br	Br	NA
5	62	CH ₂ Br	Br	H	Br	NA
6	63	-	-	-	-	NA
7	64	-	-	-	-	19%

NA = No reported activity

In an effort to elucidate the mode of action of halogenated furanones on QS systems, Zang and co-workers explored their interaction with the LuxS enzyme (Figure 4) [29]. They concluded that brominated furanones covalently modify and deactivate the LuxS enzyme, which is responsible for the production of AI-2. They also noted that an exocyclic vinyl bromide is critical for potent biological activity. Compounds **12** and **24**, which both contain a monobrominated vinyl group, inhibit LuxS to a greater extent than vinyl dibromide-containing analogues **3** and **25** (Figure 3). Defoirdt subsequently determined that **12** reduces QS bioluminescence of *V. harveyi* by decreasing the DNA-binding activity of the quorum sensing master regulator protein LuxR_{vh} [30]. In a similar vein, Manfield demonstrated that halogenated furanones cause a 100-fold reduction of the LuxR protein in *E. coli* [31].

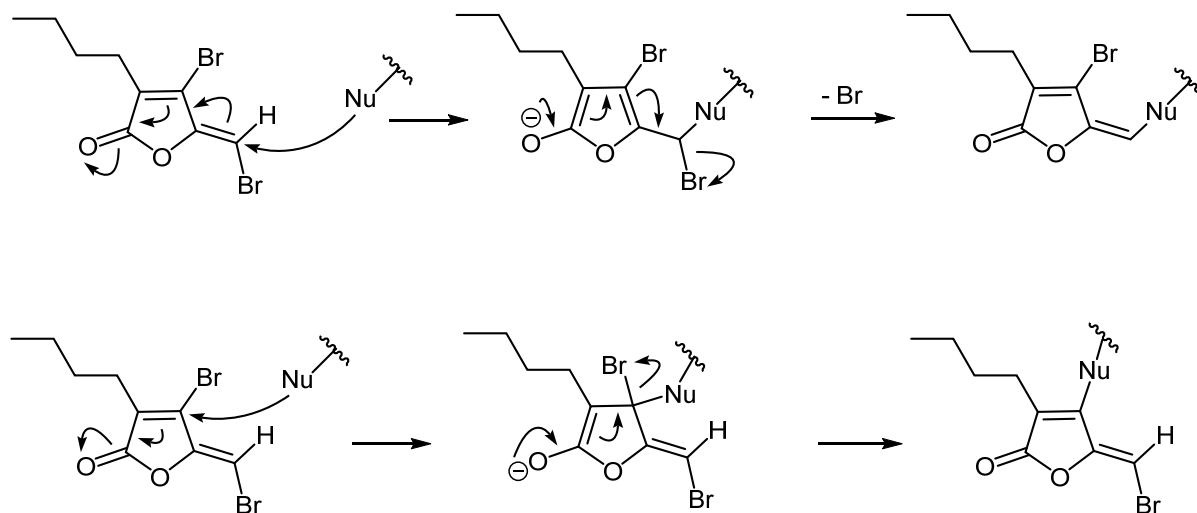


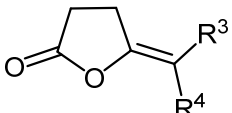
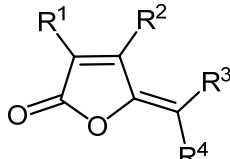
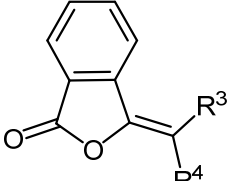
Figure 4. Proposed mode of action of brominated furanones.

In their studies of *Staphylococcus epidermidis*, Lönn-Stensrud and colleagues explored the QS properties of halogenated furanones **24** and **65-73** (Table 6) [32]. An initial bioluminescence assay

confirmed that compounds **24** (entry 1), **66** (entry 3), **70** (entry 7) and **(Z)-73** (entry 11) significantly inhibited *S. epidermidis*-induced bioluminescence. These four compounds were subsequently investigated as inhibitors of biofilm formation. Monobrominated furanone **24** proved to be the most potent compound with 68% growth inhibition (entry 1). When the exocyclic bromine in **24** was replaced with a chlorine (entry 2), the level of biofilm inhibition declined to 55%. The introduction of bromine at the C-3 position in **70** also negatively impacted on activity (entry 7). Importantly, these candidates did not influence bacterial growth, thereby underlining their potential as QS inhibitors. The authors suggest the likely mode of action of these furanones involves interruption of AI-2 communication, as adding DPD (an AI-2 precursor) counteracted the effect of the furanones.

The QS abilities of **24** against *V. harveyi* and *S. epidermidis* was also compared to several other furanones by the same researchers (Table 6) [33]. A 6.0 μ M concentration of **24** (entry 1), **67-70** (entries 4-7) and **72-73** (entries 9-11) significantly reduced *V. harveyi* BB170 bioluminescence. Compounds **67-70** and **72** inhibited bioluminescence in the range of 20% – 50%. Notably, the (*E*)- and (*Z*)-isomers of **73** had markedly different effects, with **(Z)-73** reducing bioluminescence by 60% (entry 10), while **(E)-73** reduced bioluminescence by only 30% (entry 11). **(Z)-73** proved marginally more potent than reference furanone **24**. These QS effects were not associated with a reduction in planktonic growth in either *S. epidermidis* or *V. harveyi*.

Table 6. Effect of halogenated furanones on *Staphylococcus epidermidis* and *Vibrio harveyi*.

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>65</p> </div> <div style="text-align: center;">  <p>66-72</p> </div> <div style="text-align: center;">  <p>73</p> </div> </div>								
Entry	Compound	R ¹	R ²	R ³	R ⁴	Bioluminescence reduction at 6.0 μ M (<i>S. epidermidis</i>)	Biofilm inhibition at 60 μ M (<i>S. epidermidis</i>)	Bioluminescence reduction at 6.0 μ M (<i>V. harveyi</i> BB152)
1	24	H	H	H	Br	≥55%	68%	≥60%
2	65	-	-	H	Br	≥30%	NA	NA
3	66	H	H	H	Cl	≥50%	55%	NA
4	67	CH	H	H	Br	≥20%	NA	≥25%

		3						
5	68	CH ₃	CH ₃	H	Br	≥30%	NA	≥30%
6	69	CH ₃	CH ₃	Br	H	≥20%	NA	≥20%
7	70	Br	H	H	Br	≥45%	58%	≥50%
8	71	Ph	H	Br	H	≥35%	NA	NA
9	72	Ph	H	H	Br	≥35%	NA	≥35%
10	(E)-73	-	-	Br	H	≥30%	NA	≥30%
11	(Z)-73	-	-	H	Br	≥60%	57%	≥60%

NA = No reported activity

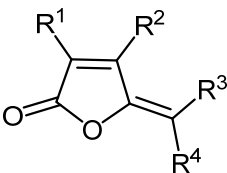
Lönn-Stensrud also discovered that **24** inhibits biofilm formation and bioluminescence induction in *Streptococcus anginosus*, *Streptococcus intermedius*, *Streptococcus mutans* and *Vibrio harveyi* BB152 [34]. Furanone-coated surfaces saw a 76% and 63% reduction in biofilm formation by *S. intermedius* and *S. mutans* respectively. The reduced biofilm formation evident at both 0.6 µM and 6.0 µM concentrations suggests that the inhibitory effect of **24** is not species specific. Additionally, a concentration of 60 µM was required before **24** interfered with streptococcal planktonic growth. Furanone **24** also reduced bioluminescence induced by *Vibrio harveyi* BB152, as well as bioluminescence induced by the cell-free supernatants of streptococci, confirming that the molecule does interact with the AI-2 QS system.

The antimicrobial potential of furanone **24**, in particular, has been well studied (Figure 3). Wu and colleagues demonstrated that **24** disrupts *E. coli* MT102 quorum sensing [35]. A similar observation was recorded for the structurally-related furanone **26** which proved active even at a ten-fold lower dose. Further analysis confirmed that **26** also regulates QS gene expression in *P. aeruginosa* PAO1 in a dose dependent manner. A series of comprehensive *in vivo* studies with mouse lung infection models indicated that administration **24** or **26** was associated with improved survival rates and better bacterial clearance. **24** and **26** were shown to interfere with the QS molecule *N*-acyl homoserine lactone, which resulted in reduced severity of infection and increased bacterial clearance in the lungs of mice infected with *P. aeruginosa*. Mice infected with *P. aeruginosa* survived

significantly longer when treated with these furanones. Furanone **26** was also found to increase the clearance of *P. aeruginosa* in a foreign body infection model [36]. Silicone implants colonised with a wild type *P. aeruginosa* biofilm were inserted into mice which were subsequently treated with a combination of QSI **26** and tobramycin. The dual treatment saw a reduction in virulence on treatment with QS inhibitor **26** prior to administration of the antibiotic. Mouse pulmonary infection models undertaken by Hentzer and co-workers further highlighted the QS inhibitory potential of these brominated furanones against *P. aeruginosa* [37].

Kumar *et al.* evaluated the QS inhibitory activity of alkyne-containing furanones derived from precursor **26** (Table 7, entry 1) against the *P. aeruginosa* MH602 *lasB* reporter strain [38]. This study established that, with the exception of **83** (entry 11), a bromine substituent is required for potent QS inhibition. Compounds **74-77** (entries 2-5) and **78-81** (entries 6-9) which contain aryl or alkyl groups in place of bromine, exhibited poor activity and typically inhibited QS by less than 30% at 250 μ M concentrations. Better activity was observed with **82**, which contains a cyclohexyl substituent, and inhibited QS by more than 50% at 250 μ M concentration (entry 10). Brominated derivative **84** (entry 12) was the most potent candidate tested (97.6% QS inhibition) and compared favourably with structurally similar **83** (82.2% QS inhibition, entry 11) as well as the study control **26** (89.7% QS inhibition). Although **83** was less potent than **26** or **84**, it was also less bactericidal, and may therefore constitute a more useful QSI.

Table 7. QS inhibition against *Pseudomonas aeruginosa* MH602 *lasB* reporter strain at 250 μ M.

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Entry	Compound	R ¹	R ²	R ³	R ⁴	Quorum Sensing Inhibition
1	26	H	Br	H	Br	89.7 \pm 4.6%
2	74	H	C ₆ H ₅ -C \equiv C-	H	C ₆ H ₄ -C \equiv C-	13.3 \pm 0.1%
3	75	H	<i>p</i> -MeC ₆ H ₄ -C \equiv C-	H	<i>p</i> -MeC ₆ H ₄ -C \equiv C-	11.2 \pm 1.2%
4	76	H	<i>t</i> BuC ₆ H ₄ -C \equiv C-	H	<i>t</i> BuC ₆ H ₄	13.2 \pm 1.4%
5	77	H	cyclohexyl-C \equiv C-	H	cyclohexyl-C \equiv C-	18.2 \pm 2.0%

6	78	Et	HC≡C-	H	HC≡C-	31.8 ± 1.2%
7	79	Et	C ₆ H ₅ -C≡C-	H	PhC≡C-	8.1 ± 2.2%
8	80	Et	<i>p</i> -MeC ₆ H ₄ -C≡C-	H	<i>p</i> -MeC ₆ H ₄ -C≡C-	3.14 ± 1.3%
9	81	Et	<i>t</i> BuC ₆ H ₄ -C≡C-	H	<i>t</i> BuC ₆ H ₄ -C≡C-	15.5 ± 1.0%
10	82	Et	cyclohexyl-C≡C-	H	cyclohexyl-C≡C-	50.6 ± 1.5%
11	83	H	HC≡C-	H	HC≡C-	82.2 ± 1.4%
12	84	H	Br	H	HC≡C-	97.6 ± 0.2%
13	85	Et	H	Br	Br	NA

NA = No reported activity

Several bicyclic furanones were investigated by Yang and co-workers as biofilm inhibitors of *Pseudomonas aeruginosa* PAO1-GFP [39]. This strain of the bacterium expresses green fluorescent proteins (GFP), allowing the bacterial biofilm to be readily visualised. The bicyclic systems containing 5-, 6- and 7-membered rings displayed differing levels of QS and biofilm inhibition, suggesting that ring size is important. Initially, the compounds were tested at 400 µM concentration to measure their impact on biofilm growth. 6-Membered ring **87** (71% inhibition) proved a more effective QSI than 5-membered **86** (50% inhibition) and 7-membered **88** (53% inhibition) (Figure 5). A subsequent dose-dependence study revealed that the larger 6- and 7-membered rings were comparable with IC₅₀ values of 145.8 µM and 139.7 µM respectively, and both were more significantly potent than the 5-membered ring (IC₅₀ ≥400 µM).

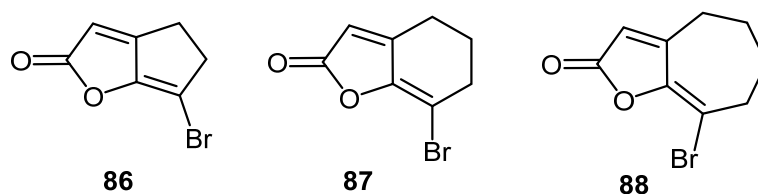


Figure 5. Bicyclic brominated furanones as potential QSIs.

Park *et al.* studied several bicyclic furanones as possible QS inhibitors of *F. nucleatum*, *P. gingivalis* and *T. forsythia* which all play a role in periodontal disease [40]. A preliminary screening identified compounds **89**, **90** and **91** as promising candidates for *F. nucleatum* biofilm inhibition (Figure 6). Surprisingly, **92**, which differs from **89** only by the presence of an additional carbon, was not

considered for further evaluation. Likewise, the incorporation of alkoxy substituents into **93a-d** did not result in increased activity.

Figure 6. Comparison of aromatic and aliphatic bicyclic furanones

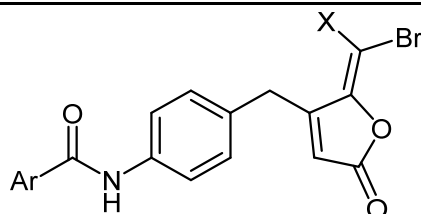
Table 8. Biofilm inhibition of furanones on *F. nucleatum*, *P. gingivalis* and *T. forsythia* at 0.002 μ M.

In a bid to develop dual OSI/anti-inflammatory conjugates, Chen introduced furanones into an amidobenzyl scaffold [41]. QS inhibition in *P. aeruginosa* was measured using QS monitors *lasB-gfp*, *rhIA-gfp* and *pqsA-gfp* (Table 9). The expression of green fluorescence proteins (GFP) by these monitors indicates the activity of the promoters *lasB*, *rhIA* and *pqsA* which play a role in QS. Fluorinated analogues were found to be the most potent with **103**, which contains an aromatic 4-

fluorobenzyl ring, inhibiting PAO1-*pqsA-gfp* by 50.29% (entry 10). Replacing the fluorine with a bromine in **109** proved detrimental, with inhibition falling to 40.75% in the *pqs* system (entry 16), while a slight increase was detected in the PAO1-*lasB-gfp* and PAO1-*rhlA-gfp* reporter strains. The introduction of electron-donating groups into **104-107** led to decreased inhibitory activity in all of the QS systems tested (entries 11-14). In general, the replacement of a vinyl bromide with hydrogen was associated with reduced inhibition in the *pqs* and *lasB* systems. The opposite proved true in the *rhlA* system. Additional experimentation established **103** as an excellent, dual-action anti-inflammatory agent, most likely *via* its interaction with peroxisome proliferator-activated receptor γ (PPAR γ) which controls the expression of pro-inflammatory genes such as TNF- α and interleukin-6.

Table 9. QS activity of arylamidobenzyl-substituted furanones against *P. aeruginosa* at 10 μ M.

Entry	Compound	X	Ar	Quorum sensing inhibition		
				PAO1- <i>lasB-gfp</i>	PAO1- <i>rhlA-gfp</i>	PAO1- <i>pqsA-gfp</i>
1	94	Br	Ph-	21.19 \pm 0.41%	20.25 \pm 4.34%	28.23 \pm 0.87%
2	95	H	Ph-	16.13 \pm 0.40 %	17.20 \pm 2.47%	34.54 \pm 3.20%

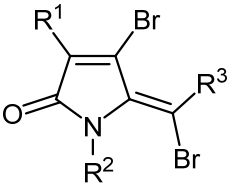


3	96	Br	2-Furanyl-	22.89 ± 0.31%	23.57 ± 4.57%	34.64 ± 4.04%
4	97	H	2-Furanyl-	17.33 ± 0.81%	13.64 ± 2.48%	20.37 ± 3.21%
5	98	Br	2-Thienyl-	19.91 ± 5.04%	19.57 ± 3.65%	26.44 ± 1.91%
6	99	H	2-Thienyl-	10.22 ± 0.92%	23.93 ± 2.20%	20.33 ± 1.02%
7	100	Br	Bn-	19.21 ± 1.01%	19.22 ± 2.93%	24.83 ± 2.00%
8	101	H	Bn-	18.01 ± 2.92%	17.28 ± 1.82%	25.82 ± 1.72%
9	102	Br	4-F-C ₆ H ₄ -CH ₂ -	9.49 ± 3.29%	15.93 ± 0.92%	19.99 ± 3.01%
10	103	H	4-F-C ₆ H ₄ -CH ₂ -	15.20 ± 2.05%	14.71 ± 4.97%	50.29 ± 1.07%
11	104	Br	4-MeO-C ₆ H ₄ -CH ₂ -	11.76 ± 2.29%	13.77 ± 4.27%	20.55 ± 1.15%
12	105	H	4-MeO-C ₆ H ₄ -CH ₂ -	14.75 ± 0.37%	11.81 ± 3.49%	26.44 ± 1.91%
13	106	Br	4-Me-C ₆ H ₄ -CH ₂ -	14.22 ± 0.47%	16.37 ± 3.30%	24.50 ± 2.86%
14	107	H	4-Me-C ₆ H ₄ -CH ₂ -	19.02 ± 4.92%	18.22 ± 3.92%	29.83 ± 1.02%
15	108	Br	4-Br-C ₆ H ₄ -CH ₂ -	10.41 ± 2.38%	20.01 ± 2.49%	27.92 ± 2.93%
16	109	H	4-Br-C ₆ H ₄ -CH ₂ -	24.87 ± 4.61%	17.17 ± 3.87%	40.75 ± 6.42%
17	110	Br	2,6-(Cl) ₂ -C ₆ H ₃ -CH ₂ -	19.13 ± 2.49%	19.20 ± 2.30%	19.29 ± 2.29%
18	111	H	2,6-(Cl) ₂ -C ₆ H ₃ -CH ₂ -	16.91 ± 4.51%	15.17 ± 2.88%	17.62 ± 2.53%
19	112	Br	C ₆ H ₅ -(CH ₂) ₂ -	11.01 ± 3.21%	19.50 ± 2.63%	22.89 ± 0.25%
20	113	H	Ph-(CH ₂) ₂ -	14.94 ± 0.01%	15.25 ± 3.61%	20.60 ± 1.20%
21	114	Br	Ph-CH=CH-	9.48 ± 5.47%	18.07 ± 7.62%	16.22 ± 0.19%
22	115	H	Ph-CH=CH-	11.84 ± 4.32%	12.02 ± 1.09%	23.09 ± 1.44%
23	116	Br	4-MeO-C ₆ H ₄ -CH=CH-	5.03 ± 0.92%	12.93 ± 1.48%	21.92 ± 3.99%
24	117	H	4-MeO-C ₆ H ₄ -CH=CH-	12.23 ± 6.66%	10.56 ± 2.01%	24.30 ± 1.17%
25	118	Br	3,4,5-(MeO) ₃ -C ₆ H ₄ -CH ₂ -	9.79 ± 1.17%	7.66 ± 5.99%	19.53 ± 3.11%
26	119	H	3,4,5-(MeO) ₃ -C ₆ H ₄ -CH ₂ -	8.53 ± 0.79%	5.57 ± 3.44%	20.28 ± 2.37%

3. Dihydropyrrolones

The replacement of oxygen in the furanone ring with nitrogen was investigated by Kumar and co-workers in their search for novel antimicrobials [42, 43]. The resultant 1,5-dihydropyrrol-2-ones are generally non-cytotoxic to mammalian cell lines and hydrolytically more stable than furanones (Table 10). Analysis of the structure activity relationships of the dihydropyrrolones suggests that the absence of an alkyl group at the C3 position (entries 1-7) results in increased inhibition in *E. coli* compared to the C3-alkylated derivatives (entries 8-13) [42]. The non-alkylated derivatives varied from being inactive to having AIC₄₀ values of 19-26. AIC₄₀ is the ratio of synthetic inhibitor to natural AHL signalling molecule that is required to lower GFP expression to 40%. The authors also discovered that while variation of the nitrogen substituent did affect activity, no obvious trend was apparent. Incorporating a second bromine produced a range of dibromomethylene analogues (entries 14-19) that were less potent than the structurally similar bromomethylene derivatives (entries 1-13). For example, *N*-benzyl-substituted **121**, with an AIC₄₀ of 26 (entry 2), was five times more active than dibromomethylene-containing **133** (entry 14). Similarly, **134** (entry 15) and **136** (entry 17) were inactive despite the matching bromomethylene derivatives **122** and **124** recording AIC₄₀ values of 19 (entry 3) and 20 (entry 5) respectively.

Table 10. AIC₄₀ values of 1,5-dihydropyrrol-2-ones against *Escherichia coli*.

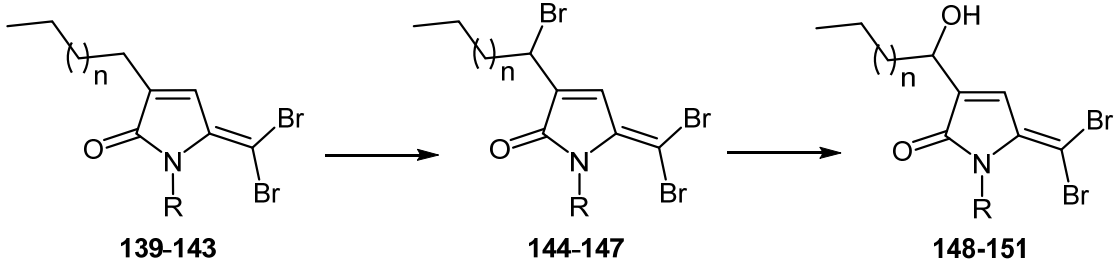
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Entry	Compound	R ¹	R ²	R ³	AIC ₄₀
1	120	H	Ph	H	NA
2	121	H	Bn	H	26
3	122	H	<i>n</i> -Bu	H	19
4	123	H	<i>n</i> -Hex	H	NA
5	124	H	<i>n</i> -Oct	H	20
6	125	H	<i>n</i> -Dodecyl	H	NA
7	126	H	<i>i</i> -Bu	H	NA
8	127	<i>n</i> -Bu	Ph	H	NA
9	128	<i>n</i> -Bu	Bn	H	NA

10	129	<i>n</i> -Bu	<i>n</i> -Bu	H	279
11	130	<i>n</i> -Hex	Ph	H	NA
12	131	<i>n</i> -Hex	Bn	H	NA
13	132	<i>n</i> -Hex	<i>n</i> -Bu	H	NA
14	133	H	Bn	Br	150
15	134	H	<i>n</i> -Bu	Br	NA
16	135	H	<i>n</i> -Hex	Br	NA
17	136	H	<i>n</i> -Oct	Br	NA
18	137	H	<i>n</i> -Dodecyl	Br	NA
19	138	H	<i>i</i> -Pr	Br	65

NA = No reported activity

A related series of C3-alkylated dihydropyrrolones displayed similarly low levels of QS inhibition (Table 11) [42]. Increasing or decreasing the length of the C3-alkyl chain produced a different outcome depending on the nitrogen substituent. *N*-Phenyl-substituted **139** (entry 1), incorporating a butyl chain at C3, recorded an AIC₄₀ value of 97 and was more active than hexyl-containing **141** (entry 3). By contrast, *N*-benzyl-substituted analogues displayed the opposite trend and activity increased as the alkyl chain was lengthened from 4 to 6 carbons, with AIC₄₀ values of 220 (entry 2) and 140 (entry 4) respectively. The presence of α -substituents (e.g. Br or OH) on the C3-alkyl chain proved inconsequential and these analogues were generally devoid of activity (entries 7-13). However, one particular α -brominated derivative **144** was surprisingly potent (entry 6), with the lowest overall AIC₄₀ of 1.95. While these 1,5-dihydropyrrol-2-ones proved generally less effective than naturally-occurring fimbrolides, they still possess significant potential as QS inhibitors given their low toxicities.

Table 11. AIC₄₀ values of C3-alkylated 1,5-dihydropyrrol-2-ones.

				
Entry	Compound	R	n	AIC ₄₀
1	139	Ph	1	97
2	140	Bn	1	220
3	141	Ph	3	248
4	142	Bn	3	140
5	143	<i>n</i> -Bu	3	NA
6	144	Ph	1	1.95
7	145	Bn	1	NA
8	146	Ph	3	NA
9	147	Bn	3	NA
10	148	Ph	1	NA
11	149	Bn	1	NA
12	150	Ph	3	NA
13	151	Bn	3	NA

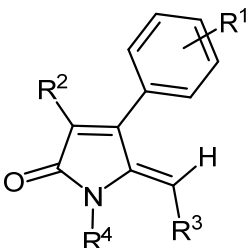
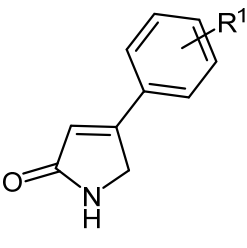
NA = No reported activity

More recently, Kumar and colleagues synthesised a library of arylated dihydropyrrolones as potential QS inhibitors of *P. aeruginosa* [8]. The various structure activity relationships were uncovered by altering the substituents at both the nitrogen and C-3, by incorporating different aromatic substituents at C-4 and by modifying the exocyclic vinyl group at C-5 (Table 12). Phenyl-substituted **152** acted as a reference compound, inhibiting QS activity by 19.6% at 31.25 μ M concentration and biofilm formation by 47.65% at 250 μ M concentration (entry 1). **153-161** (entries 2-10) and **163-164** (entries 12-13) bearing halogen, nitro, methoxy and alkyl substituents exhibited higher QSI activity (29.6% - 63.1%) than unsubstituted **152**. Carboxylic acid derivative **162** (entry 11)

was a comparable QS inhibitor to **152** but a significantly poorer inhibitor of biofilm growth. *para*-Brominated **160** was the most active analogue with QS inhibitory activity of 63.1% (entry 9). Changing the ring substitution pattern had little impact on activity with the 2-, 3- or 4-substituted fluorinated or chlorinated analogues returning inhibition values in the range of 33.3% – 39.5% (entries 2-4) and 26.3% – 37.8% (entries 5-7) respectively.

The introduction of a methyl group to the 3-position of the dihydropyrrolones was unfavourable (entries 15-19) compared to their non-alkylated analogues **153** (entry 2), **155** (entry 4), or **158-160** (entries 7-9). These findings suggest that the presence of a hydrogen at the C3 position represents a critical feature. Additionally, the introduction of substituted aromatic or heterocyclic groups at the nitrogen was associated with reduced potency (entries 31-44). The comparison of **171-173** with **196-203** sheds some light on the importance of the exocyclic vinyl group for QSI activity in *P. aeruginosa*. Dihydropyrrolones **171-173** all feature a bromine atom on the exocyclic vinyl group and displayed relatively high QSI activity of 38.2%–61.5% (entries 20-22). By contrast, analogues **196-203**, which lacked an exocyclic vinyl group, returned a reduced QS response ranging from 21.9% to 27.1% (entries 45-52). **171-173** also inhibit bacterial growth by over 30% at 125 μ M concentration, suggesting that the presence of a vinylic bromine is toxic to the bacteria.

Table 12. Biofilm and QS inhibition of 1,5-dihydropyrrol-2-ones against *P. aeruginosa*.

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>152-195</p> </div> <div style="text-align: center;">  <p>196-203</p> </div> </div>							
Entry	Compound	R ¹	R ²	R ³	R ⁴	Biofilm Inhibition (250 μ M)	QS Inhibition (31.25 μ M)
1	152	H	H	H	H	47.6 \pm 3.8%	19.6 \pm 4.8%
2	153	2-F	H	H	H	51.3 \pm 4.7%	37.8 \pm 1.6%

3	154	3-F	H	H	H	55.2 ± 3.1%	39.5 ± 0.2%
4	155	4-F	H	H	H	46.1 ± 5.3%	33.3 ± 1.3%
5	156	2-Cl	H	H	H	NA	37.8 ± 1.6%
6	157	3-Cl	H	H	H	47.3 ± 1.9%	26.3 ± 5.2%
7	158	4-Cl	H	H	H	60.9 ± 3.4%	33.3 ± 1.3%
8	159	3-CF ₃	H	H	H	50.3 ± 4.9%	35.4 ± 3.2%
9	160	4-Br	H	H	H	62.5 ± 2.7%	63.1 ± 4.7%
10	161	4-NO ₂	H	H	H	57.2 ± 4.1%	51.3 ± 3.0%
11	162	4-HO ₂ C	H	H	H	12.9 ± 4.5%	19.6 ± 8.7%
12	163	4-MeO	H	H	H	38.8 ± 6.0%	37.0 ± 5.4%
13	164	CH ₃	H	H	H	NA	36.5 ± 3.8%
14	165	CH ₃ CH ₂	H	H	H	38.9 ± 7.8%	32.5 ± 1.9%
15	166	4-F	CH ₃	H	H	4.4 ± 3.4%	26.3 ± 4.0%
16	167	2-F	CH ₃	H	H	NA	28.8 ± 1.2%
17	168	4-Cl	CH ₃	H	H	NA	23.7 ± 2.2%
18	169	4-Br	CH ₃	H	H	NA	37.7 ± 3.5%
19	170	3-CF ₃	CH ₃	H	H	NA	26.3 ± 2.3%
20	171	H	H	Br	H	NA	56.8 ± 0.7%
21	172	2-F	H	Br	H	NA	61.5 ± 0.0%
22	173	4-Br	H	Br	H	NA	38.2 ± 2.3%
23	174	2-F	H	H	Ph	38.2 ± 3.0%	46.4 ± 1.0%
24	175	2-F	H	H	3-F-C ₆ H ₄	58.9 ± 4.8%	24.0 ± 5.8%
25	176	2-F	H	H	3-Cl-C ₆ H ₄	46.9 ± 0.44%	38.7 ± 2.2%
26	177	2-F	H	H	4-Cl-C ₆ H ₄	46.5 ± 2.6%	36.2 ± 5.3%
27	178	2-F	H	H	3-Br-C ₆ H ₄	40.8 ± 3.2%	29.3 ± 2.6%
28	179	2-F	H	H	4-Br-C ₆ H ₄	49.2 ± 0.4%	39.7 ± 6.6%
29	180	2-F	H	H	3-CH ₃ -C ₆ H ₄	42.0 ± 4.3%	25.3 ± 2.1%

30	181	2-F	H	H	4-CH ₃ -C ₆ H ₄	49.3 ± 2.0%	29.6 ± 5.0%
31	182	4-Br	H	H	Ph	35.2 ± 1.8%	22.0 ± 6.4%
32	183	4-Br	H	H	3-F-C ₆ H ₄	29.3 ± 2.4%	5.80 ± 2.94%
33	184	4-Br	H	H	4-F-C ₆ H ₄	27.7 ± 0.8%	9.23 ± 5.19%
34	185	4-Br	H	H	4-Cl-C ₆ H ₄	43.8 ± 2.0%	7.39 ± 1.66%
35	186	4-Br	H	H	3-Br-C ₆ H ₄	47.1 ± 0.4%	12.6 ± 5.7%
36	187	4-Br	H	H	4-Br-C ₆ H ₄	17.0 ± 5.2%	8.81 ± 4.34%
37	188	4-Br	H	H	3-CH ₃ -C ₆ H ₄	16.3 ± 1.3%	15.2 ± 7.6%
38	189	4-Br	H	H	4-CH ₃ O-C ₆ H ₄	54.9 ± 4.7%	14.4 ± 1.0%
39	190	4-Br	H	H	4-CF ₃ -C ₆ H ₄	37.5 ± 1.2%	11.2 ± 1.5%
40	191	4-Br	H	H	4-MeSO ₂ -C ₆ H ₄	10.3 ± 3.4%	11.2 ± 2.2%
41	192	4-Br	H	H	3-Thienyl	87.4 ± 2.6%	49.9 ± 3.8%
42	193	4-Br	H	H	3-benzothiophenyl	13.2 ± 3.0%	34.1 ± 2.3%
43	194	2-F	H	H	<i>n</i> -Bu	52.2 ± 4.7%	38.2 ± 4.4%
44	195	2-F	H	H	Bn	NA	27.0 ± 6.1%
45	196	H	-	-	-	14.4 ± 2.0%	31.7 ± 4.6%
46	197	4-F	-	-	-	NA	27.1 ± 6.8%
47	198	3-F	-	-	-	21.4 ± 2.8%	22.4 ± 7.5%
48	199	2-F	-	-	-	10.9 ± 4.3%	24.4 ± 1.2%
49	200	4-Cl	-	-	-	9.6 ± 5.6%	22.6 ± 1.8%
50	201	4-Br	-	-	-	NA	21.9 ± 1.7%
51	202	4-MeO	-	-	-	NA	26.9 ± 0.4%
52	203	3-thienyl	-	-	-	87.9 ± 3.1%	24.7 ± 2.9%

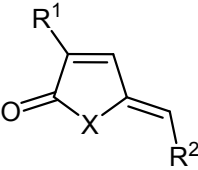
NA = No reported activity

In related work, the same authors attached similar dihydropyrrolones to a variety of different surfaces and successfully demonstrated that these compounds are effective in preventing *in vitro* *P. aeruginosa* [44] and *in vivo* *S. aureus* [45] colonisation.

4. Thiophenones

The strategy of replacing oxygen with sulfur has a well-established track record in medicinal chemistry [46]. Bennneche and co-workers recently investigated thiophenones as sulfur-containing bioisosteres of furanones (Table 13) [47]. Substitution of the ring oxygen with sulfur afforded more potent biofilm inhibitors of *Vibrio harveyi* BB120. At 20 μM concentration, **205** reduced bacterial biofilm formation by 50% (entry 2), while a 50 μM concentration of **24** was required for the same effect (entry 13). Replacing the chlorine atom in **204** (entry 1) with a bromine proved beneficial (entry 2). An additional increase in potency was observed on introduction of a bulkier iodine atom, with **206** recording a biofilm inhibitory concentration (BIC_{50}) of less than 5 μM (entry 3). BIC_{50} is the concentration of a compound required to inhibit biofilm formation by 50%. The presence of chloromethyl group at C3 resulted in a halving of BIC_{50} from 20 μM (entry 2) to approximately 10 μM (entry 7). Apart from **210** (entry 7), C3-substitution generally had an overall negative effect (entries 8-12). This was especially true in the case of bulky alkyl groups which were associated with a near total loss of biological activity (entry 9). A general trend was observed where those candidates which successfully inhibited biofilm growth also inhibited planktonic growth, albeit to a lesser extent.

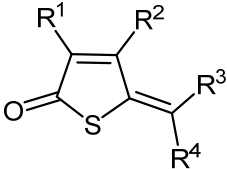
Table 13. Effect of thiophenones on *Vibrio harveyi* BB120.

					
Entry	Compound	R ¹	X	R ²	BIC ₅₀ (μM)
1	204	H	S	Cl	≥ 20
2	205	H	S	Br	20
3	206	H	S	I	≤ 5
4	207	H	S	N ₃	35
5	208	H	S	SCN	7
6	209	Et	S	Br	15.5
7	210	CH ₂ Cl	S	Br	≥ 10
8	211	<i>i</i> Pr	S	Br	37

9	212	<i>t</i> Bu	S	Br	≥300
10	213	Br	S	Cl	61
11	214	Br	S	SCN	20
12	215	2-thienyl	S	Br	50
13	24	H	O	Br	≥50

A series of more elaborate thiophenones was subsequently prepared by Benneche who discovered that fifteen of the twenty compounds assayed inhibited wild *type V. harveyi* bioluminescence at 0.25 μ M concentration (Table 14) [48]. Chlorothiophenone **204** (entry 1) and iodothiophenone **206** (entry 2) were found to possess low specific QS inhibitory activity (A_{QSI}) of <1, with the chlorine derivative proving slightly more potent. A_{QSI} is the ratio between inhibition of QS-regulated activity in a reporter strain and inhibition of the same activity when it is independent of QS. Six compounds recorded a relatively high specific QS activity of >10. Of these, a bromomethylene side chain was common to **219** (entry 6), **221** (entry 8), **225** (entry 12) and **231** (entry 18). By contrast, **223** (entry 10) and **227** (entry 14) did not feature a similar bromomethylene motif, suggesting that it is not necessarily a prerequisite for activity.

Table 14. Effect of thiophenones on wild *type Vibrio harveyi*.

						
Entry	Compound	R ¹	R ²	R ³	R ⁴	A_{QSI}
1	204	H	H	H	Cl	0.7
2	206	H	H	H	I	0.2
3	216	H	H	H	4-NO ₂ -C ₆ H ₄ -O-	0.3
4	217	H	H	H	Pyr-S-	NS
5	218	H	H	H	4-HO-C ₆ H ₄ -S-	7.0
6	219	H	H	Ph	Br	17.0
7	220	Br	H	H	Br	NS

8	221	HOCH ₂	H	H	Br	33.0
9	222	(CH ₃) ₃ C	H	H	Br	3.7
10	223	HOCH ₂	H	H	Ph-S-	20.0
11	224	CH ₃	H	H	Br	4.3
12	225	CH ₃ CO ₂ CH ₂ -	H	H	Br	10.3
13	226	-CH ₂ -O ₂ C(CH)CH ₂ -	H	H	Br	3.0
14	227	Cl	H	H	CH ₃ BrC=CH-	19.0
15	228	HOCH ₂	H	H	CH ₃ S	NS
16	229	CH ₃ CH(OH)	H	H	Br	3.2
17	230	CH ₃ CHBr	H	H	Br	3.3
18	231	H	Br	H	Br	17.0

NS = no significant inhibition of QS-regulated bioluminescence in *V. harveyi*.

In a follow-on study, carboxylic acid-containing **232** was compared to thiophenone **205** as a potential biofilm inhibitor of *S. epidermidis* ATCC 35984 (Figure 7) [49]. Addition of 5 μ M **205** or **232** reduced biofilm growth by 56% and 59% respectively, without impacting on bacterial growth. In related work, Benneche and colleagues demonstrated that QS-regulated *V. harveyi* BB120 bioluminescence was reduced by 50% in the presence of 2.5 μ M of novel thiophenone **232** [50]. **232** exhibited very limited toxicity, even at higher concentrations, in contrast to **205**, presumably due to the presence of the carboxylic side chain. The authors surmise that the presence of the carboxylic acid side chain hinders the unwanted reaction of **232** with nucleophilic amino acid residues of essential proteins.

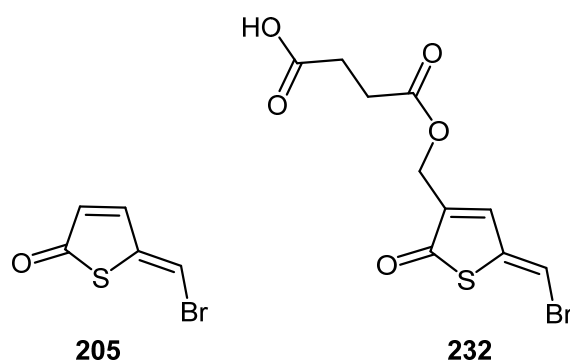


Figure 7. Novel thiophenones as potential QS inhibitors.

5. Conclusion

In this review, we have outlined the structure activity relationships of halogenated furanones as well as related dihydropyrrolones and thiophenones as potential inhibitors of bacterial quorum sensing. It is clear from the findings presented here that such small molecule inhibitors are effective against a wide range of pathogenic Gram positive and Gram negative bacteria, including *P. aeruginosa*, *S. aureus* and *S. typhimurium* amongst others. Importantly, many of these inhibitors effectively reduce bacterial pathogenicity without impacting on bacterial growth, thereby reducing evolutionary pressure towards new resistance mechanisms. The intricate structural activity relationships of these compounds have been described, laying the foundation for the generation of ever more potent antimicrobials.

6. Future Perspective

Antimicrobial resistance has been identified as a global threat to human health. Over 750,000 deaths a year are caused by bacterial resistance, and the United Nations has estimated that this number could grow to 10 million deaths annually by 2050. With the well-known difficulties in both discovering new classes of antibiotics as well as subsequently bringing them to market, imaginative strategies are urgently required. The development of novel quorum sensing inhibitors represents one promising approach. By developing molecules that disrupt bacterial communication pathways, existing antibiotics, which were previously considered redundant, could be recovered when co-administered with these potent quorum sensing inhibitors. Such an approach would greatly increase the arsenal of effective antibiotics available to clinicians in tackling resistant infections. Furthermore, such an approach would be highly commercially attractive to the pharmaceutical industry.

Although several studies have demonstrated how halogenated furanones, dihydropyrrolones and thiophenones reduce bacterial pathogenicity, clinical trials are still necessary to assess their efficacy as QS inhibitors. To date, only a small number of clinical trials have been conducted on QS inhibitors, with all of the molecules investigated having been previously approved by the FDA [7]. For example, the macrolide antibiotic azithromycin was found to display QS-dependent virulence inhibition *in vitro* over several studies [51-55]. These findings paved the way for a randomized, double-blind, multicentre trial, in which azithromycin was found to reduce QS-regulated virulence in patients colonised with *P. aeruginosa* [56]. The incidence of ventilator-associated pneumonia, which results from *P. aeruginosa* colonisation, was reduced when patients were treated with azithromycin (300

mg/day). Of particular note, rhamnolipids, which are considered to be QS-regulated virulence factors, were significantly lowered in the presence of azithromycin.

No novel compounds capable of interfering with biofilm formation or quorum sensing have yet been clinically approved solely for that purpose. Several FDA-approved drugs have, however, been repurposed as potential anti-virulence agents [57, 58]. Mitomycin C suppresses biofilm formation in *E. coli*, *P. aeruginosa* and *S. aureus* [59]. Fluorouracil (5-FU) inhibits biofilm formation in *E. coli* [60] and *S. epidermidis* [61] and inhibits both biofilm formation and quorum sensing in *P. aeruginosa* [62]. Azacitidine, toremifene and aminolevulinic acid have also been shown to possess anti-biofilm potential against *S. pneumoniae* [63], *S. aureus* [64] and *S. epidermidis* [65] respectively. The ability of *N*-acetylcysteine to interfere with *H. pylori* biofilms was similarly assessed in a randomized trial by Cammarota and co-workers [66]. When administered prior to a culture-guided antibiotic regime, *N*-acetylcysteine increased the eradication of *H. pylori* in patients with a history of previous treatment failures. Patients who were administered *N*-acetylcysteine prior to treatment with antibiotics saw a 65% *H. pylori* eradication rate, while the pathogen was eradicated in only 20% of patients who received no *N*-acetylcysteine. Research conducted by Zala, Karbasi and Gurbuz also confirmed that *H. pylori* clearance was increased when patients were treated with both *N*-acetylcysteine and an antibiotic regime [67-69]. Such studies highlight the clinical potential of both anti-biofilm agents and quorum sensing inhibitors in the race to overcome antibiotic resistance.

7. Summary Points

Background:

- Quorum sensing (QS) is a bacterial cell-cell communication mechanism that allows bacteria to regulate gene expression in response to changes in population density.

- Furanones isolated from marine alga *Delisea pulchra* have been identified as potential QS inhibitors, in particular the AI-2 signalling system.

Furanones:

- A wide variety of synthetic furanones have been developed, with many exhibiting potent QS effects.

- The activity of these compounds is highly dependent on the nature of the ring substituents, with different structure activity relationships at play in different bacterial strains.

Dihydropyrrolones:

-Dihydropyrrolones are generally less active than the corresponding furanone analogues.

-However, their reduced toxicity suggests they may constitute more useful QS inhibitors.

Thiophenones

-Thiophenones are sulfur-based isosteres of the naturally-occurring furanone lead compounds.

-In specific strains, thiophenones outperform their furanone analogues, both in terms of activity and safety.

Financial and competing interests disclosure

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